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Case Report

Improbable Flow Cytometric Measurements

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Abstract

Here we report an exceptional observation in clinical practice: the occurrence of strictly similar cytometry measurements involving five different parameters on two distinct blood samples collected seven months apart from the same HIV-infected patient.

Keywords: cytometry; Follow up HIV therapy; CD4 count

Introduction

Quantification of lymphocyte subpopulations by flow cytometry is routinely used to appreciate immune restoration induced by antiretroviral therapy (ART) in HIV-infected subjects and to estimate the capacity of the patient to mount an immune response [1]. Assessment of CD4 T cells by flow cytometry is automated, robust and has intra-run variation coefficient of approximately 1 to 3% [2]. However, CD4 T cell count is subject to significant intra-individual variability due to circadian changes in healthy individuals, and to factors such as treatments and comorbidities in persons living with HIV. Variation thresholds as high as 50% and 6.4% have been proposed for declines of CD4 T cells counts and percentage, respectively [2]. Here, we report and comment the improbable case of strictly identical flow cytometry measurement results involving several parameters on two distinct blood samples collected seven months apart from the same patient.

Materials and methods

The patient was a 60 years old HIV-infected man followed in the Department of Infectious Diseases of the University Teaching Hospital (CHU) in Montpellier, France for long-term ART (dolutegravir/abacavir/lamivudine). He was asymptomatic and considered as on fully suppressive therapy for several years. CD4 T cells were enumerated using four colours staining with a panel of antibodies specific for CD3, CD4, CD8, CD45, (CYTO-STAT1/tetra-CHROM™, Beckmann Coulter) on an automated flow cytometer (Navios, Beckman Coulter). The lymphocyte counts and lymphocyte subsets were enumerated at the last four 6- monthly visits, as displayed in the Table 1.

Date of sampling/analysis	Reference range	12 May 2020	13 October 2020	11 May 2021	26 October 2021
Lymphocytes count, cell/mm3	1500-4000 cells/ mm3	1512	2178	2178	1628
Lymphocytes TCD3+, %	60-80%	52	55	55	56
Lymphocytes TCD3+, n/mm3	600-2100 cells/ mm3	786	1198	1198	911
Lymphocytes TCD4+, %	30-45%	28	31	31	31
Lymphocytes TCD4+, n/mm3	690-1200 cells/ mm3	423	675	675	504
Lymphocytes TCD8+, %	20-35%	23	22	22	24
Lymphocytes TCD8+, n/mm3	390-820 cells/mm3	348	479	479	391
Ratio CD4+/CD8+	0.9-2.0	1.2	1.4	1.4	1.3
Lymphocytes TCD8+CD38+/ CD8+, %		32	44	49	ND
Lymphocyte CD8+CD38++, %	1-7%	1.9	5.8	8.5	ND

ND=Not done

Table 1: Lymphocyte count and T cell subsets enumeration on four consecutive blood samples collected six months apart from the same patient.

Results

On October 13, 2020 and on May 11, 2021, not only were the lymphocyte counts strictly identical but lymphocytes subsets in percentages were also identical although strikingly different from the previous (12 May 2020) and the next (26 October 2021) six-month samples. A notable exception consisted of the activated CD8 T cells, which differed in the last two samples (although within the expected intra-laboratory variation coefficient) [3]. Based on the latter for total lymphocytes, CD3-, CD4- and CD8-T cell counts, the probability of having exactly the same results in May 2021 as in October 2020 for these 4 parameters, at the unit level, was 1.2 10-12. After verification of the validity of 11 May 2021 results, analyses were validated and notified to the practitioner and to the patient. ART was maintained unchanged. In the last two years, the automated method used for lymphocyte count [4] had not changed and the flow cytometer was used by means of exactly the same gating rules. The flow cytometer is calibrated on a monthly basis, Levey-Jennings graphs are generated and interpreted every month and an international quality assessment/quality control program (UK NEQAS) applied every other month. Cytometry analyses are routinely performed on fresh blood samples collected on EDTA tubes. The primary blood samples are discarded directly after measurements have been validated, which excludes redundant measurements on the same sample. In order to exclude artefactual observations, the screenshots of the two-flow cytometry analyses were compared (Figure 1). These graphs displayed discrete differences in cell populations' distribution that are not distinguishable based on crude enumeration data (percentages and absolute counts). We concluded that the analyses done on 13 October 2020 and on 11 May 2021 had effectively been performed on distinct blood samples and that the similarity of results is not artefactual but attributable to chance only.

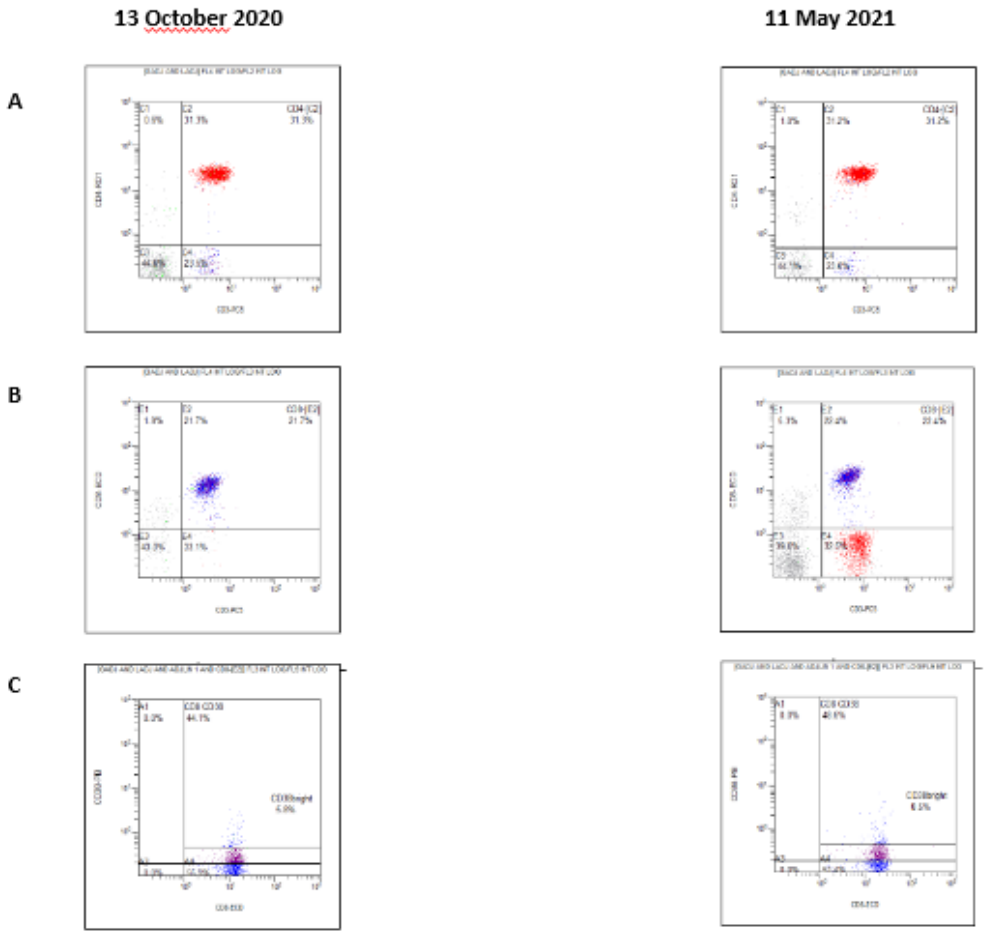

Figure 1: Phenotypic analysis of T cell subsets in blood samples collected and analysed seven-month apart. The figure consists of screenshots of the scatter plots of the following selected parameters produced by the flow cytometer.

A. CD3+CD4+

B. CD3+CD8+

C. CD8+CD38+

It shows discrete differences in cell distributions for CD3+CD4+ and CD3+CD8+ cells and a clear difference for CD8+CD38+ cells.

Discussion

Our laboratory is performing lymphocytes immune-phenotyping by flow cytometry on a routine basis on more than 60 samples a week since more than 25 years and it is the very first time that such event – two identical results involving five parameters on two separate samples collected several months apart – occurs. Based on repeatability and reproducibility assessments of the parameters' measurements (Supplemental material) and the normal value ranges, the chance of obtaining two strictly identical measurements of several parameters on two separate blood samples from the same individual is deemed infinitesimal.

Conclusion

In the case of biologic phenomenon, very improbable observation can occur and should not be a priori disregarded.

References

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Supplemental appendix

REPEATABILITY					
	Samples name	Assays (N)	Mean	Standard deviation	Variation coefficient (%)
Ly T CD3+ cells/µL	Sample 1	10	707.8	26	3.7
	Sample 2	10	2774.5	128.5	4.6
Ly T CD3+ , %	Sample 1	10	75.6	0.5	0.7
	Sample 2	10	76.2	0.5	0.6
Ly T CD3+ CD4+ cells/µL	Sample 1	10	93.8	9.3	9.9
	Sample 2	10	1529.6	84.4	5.5
Ly T CD3+ CD4+, %	Sample 1	10	10	0.7	7.3
	Sample 2	10	42	0.9	2.1
Ly T CD3+ CD8+ cells/µL	Sample 1	10	577.6	19.2	3.3
	Sample 2	10	1226.9	56.5	4.6
Ly T CD3+ CD8+, %	Sample 1	10	61.7	0.8	1.3
	Sample 2	10	33.7	0.8	2.5
REPRODUCIBILITY					
	Samples name	Assays (N)	Mean	Standard deviation	Variation coefficient (%)
Ly T CD3+ cells/µL	Immunotrol low	30	425.63	31.65	7.44
	Immunotrol	35	778.37	76.82	9.87
Ly T CD3+, %	Immunotrol low	30	54,18	1.13	2.09
	Immunotrol	35	71.7	0.72	1.01
Ly T CD3+ CD4+ cells/µL	Immunotrol low	30	130.83	11.26	8.61
	Immunotrol	35	540.63	52.62	9.73
Ly T CD3+ CD4+, %	Immunotrol low	30	16.65	0.64	3.87
	Immunotrol	35	49.82	0.7	1.4
Ly T CD3+ CD8+ cells/µL	Immunotrol low	30	269.77	21.52	7.98
	Immunotrol	35	218.11	23.52	10.78
Ly T CD3+ CD8+, %	Immunotrol low	30	34.32	0.91	2.65
	Immunotrol	35	20.07	0.46	2.28

Supplemental material: Repeatability and reproducibility assessments of the cytometry parameters measurements.